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REDUCED INOCULUM DENSITY ENHANCES SENSITIVITY OF RESISTANCE SCREENING DETECTION IN FUSIFORM RUST RESISTANCE TESTING

U.S. Forest Service
Asheville, North Carolina

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REDUCED INOCULUM DENSITY ENHANCES SENSITIVITY OF RESISTANCE SCREENING DETECTION IN FUSIFORM RUST RESISTANCE TESTING

By

Peter P. Laird[1/], John L. Knighten[2/], and Robert L. Wolfe[2/]

ABSTRACT

Reductions in inoculum density of from 150,000 spores/ml to 70,000 spores/ml for loblolly pine and from 75,000 spores/ml to 25,000 spores/ml for slash pine has enhanced the sensitivity of resistance screening detection at the Fusiform Rust Resistance Testing Facility at Asheville, N. C. Families of intermediate resistance that were previously masked using high densities are now readily apparent.

INTRODUCTION

A laboratory-greenhouse method for screening for fusiform rust resistance has been developed recently by the Southeastern Area at Bent Creek near Asheville, N. C. (Laird, Knighten, and Wolfe 1973a). However, during the original steps in the development of our testing system, it became readily apparent that we needed to develop a standardized inoculum density specifically for our system (Laird, Knighten, and Wolfe 1973a). Densities used elsewhere (Southeastern Forest Experiment Station, Athens, Ga.) were too high for our use at Bent Creek. We were getting infection levels considerably higher than desired. Known susceptible families were 95 to 100 percent infected.

Although these levels were fine for distinguishing resistant and susceptible families, we wanted a system capable of distinguishing not only resistance and susceptibility but one of delineating families of intermediate resistance as well.

1/ Plant Pathologist and 2/ Biological Laboratory Technicians, Forest Pest Management, U. S. Forest Service, Asheville, N. C.

OBJECTIVE

Our objectives here were to improve controls over inoculum density and to determine an inoculum density level capable of distinguishing susceptible intermediate and resistant families of loblolly and slash pine to fusiform rust.

GENERAL DESIGN

The availability of seed restricted the design of the evaluation. Essentially two evaluations were performed: inoculum density evaluation I with 1971 seed and II with 1972 seed. The first contained only loblolly. The second both loblolly and slash.

Each seed lot was tested twice (two runs) the runs being a month apart. Each run consisted of four replications (planter trays of 20 seedlings each). The range of densities tested for loblolly and slash were:

Loblolly	Slash
25,000 spores/ml	10,000 spores/ml
55,000 spores/ml	25,000 spores/ml
70,000 spores/ml	40,000 spores/ml
85,000 spores/ml	55,000 spores/ml
100,000 spores/ml	70,000 spores/ml
150,000 spores/ml	75,000 spores/ml

The last two densities of 150,000 for loblolly and 75,000 for slash are the densities used in our previous tests that yielded high infection rates.

Ratings of seed lot resistance are based on field progeny test information supplied to us by the seed contributors.[3/]

PROCEDURES AND EQUIPMENT

Only a brief presentation of the complex procedures and equipment is presented here. Those interested in a detailed description may refer to the literature citings or may request a copy of our laboratory guide (Laird, Knighten, and Wolfe, 1973b).

3/ Appreciation is extended to Union Camp, Westvaco, and International Paper Companies for seed contributions that made this evaluation possible.

For purposes of simplification, the procedures are described under the following headings:

Aeciospore collection and handling
Culture of oaks
Oak inoculation
Telia and basidiospore harvest
Pine inoculation
Culture of pine
Evaluation of pines for rust

With the exception of aeciospore sources used and the spray apparatus described under pine inoculation, methodology used is nearly identical to that described previously (Laird, Knighten, and Wolfe, 1973).

Aeciospore Collection and Handling

Aeciospores were collected in the field during the spring of 1972. Three inoculum sources—southern Alabama, southeastern Georgia, and a northeastern Florida source—were used for slash pine. For loblolly, a coastal South Carolina and coastal Georgia mix and a Louisiana source were used. Inoculum collections from each area consisted of a minimum of 10 galls. This insured a good representation of fungal sources from the area sampled.

The spores were collected from the galls using cyclone spore collector (Physics Shop, Iowa State Univ., Ames, Ia., Fig. 1) or by tapping the galls with a mallet after wrapping them with wax or notebook paper. Collected spores were placed immediately in ice chests. Insects were removed within 12 hours by passing the spores through a set of sieve screens. The spores were kept in ice chests or refrigerators until they were prepared for long term storage by desiccation. The spores were first dried for seven days over $CaCl_2$ in vacuum desiccators. Then, using a 72 Port Virtis manifold from a freeze dry apparatus, a vacuum desiccation system was developed where the spores were further dried and vacuum packed in glass ampoules. Roncardori and Matthews (1966) showed aeciospores can be preserved for several years with little or no loss in viability using vacuum storage techniques.

Culture of Oaks

Northern red oak (*Quercus rubra* L.) acorns were collected in the fall of 1971 and stored at cold temperatures of 1° to 2°C. When needed, the acorns were placed on vermiculite germination beds under a timed water spray system. Germinated acorns with 1- to 3-inch hypocotyls were transplanted into 5-inch diameter pots and grown in a relatively well shaded section of the greenhouse.

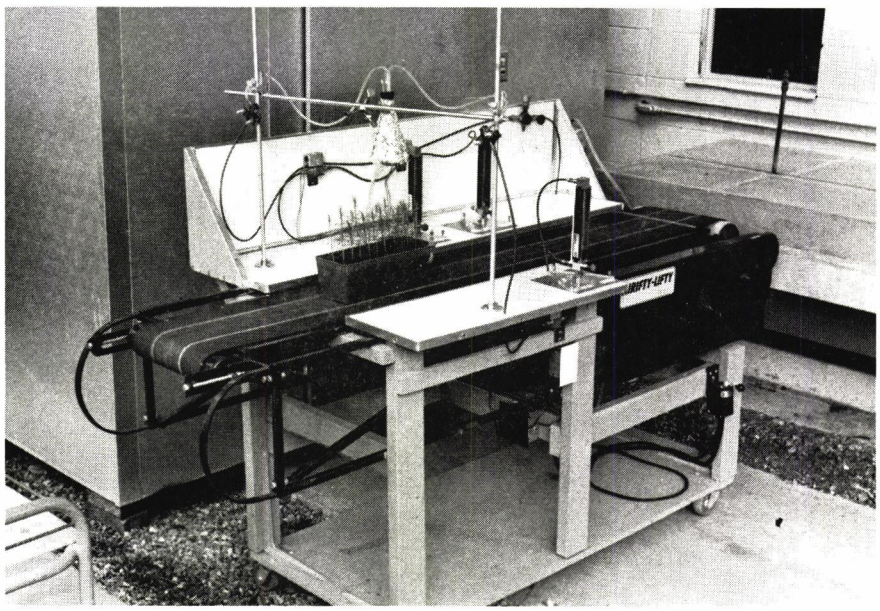

Figure 1. Mechanical spray inoculation system used to inoculate pine seedlings with Cronartium fusiforme.

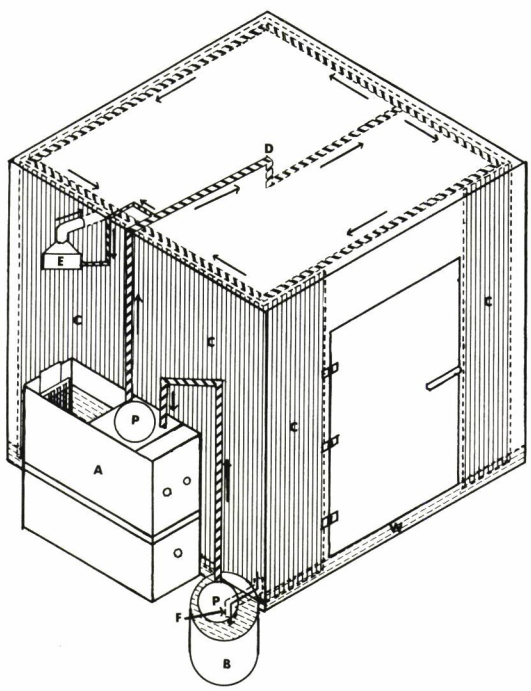

Figure 2. A schematic diagram of post inoculation incubation chamber (see text page 6 for explanation of symbols).

Oak Inoculation

Using methods modified from Nighswander and Patton (1965), Snow and Roncadori (1965), and Matthews and Rowan (1972), the oaks were inoculated with a water suspension of aeciospores. The suspension was prepared from aeciospores stored in ampoules under vacuum. The spores were first rehydrated, then placed in suspension and sprayed in a fine mist on the under surface of young tender oak leaves (14 to 20 days old).

The inoculated oaks were then incubated for 24 hours in an incubation chamber at a relative humidity of above 96 percent and temperature of 69° to 71°F. The chamber is described in detail under pine inoculation.

Telia and Basidiospore Harvest

Telial-bearing oak leaves were harvested two weeks after the telia first appeared. According to Powers and Roncadori (1966), maximum basidiospore harvest is attained at this age. Basidiospores were harvested following procedures similar to those of Matthews et al (1971) by placing the detached leaves stapled to filter paper on the inner surface lid of humidity chambers and incubating for 48 hours at 20°C. so that as the spores are released from the telial columns they fall into acidified water (pH2) at the bottom of the chamber. The low pH inhibits spore germination and permits the spores to be harvested at the end of a 48-hour period instead of every few hours. A millipore filter system was employed to collect the basidiospores from the acidified water on filter discs (Miller, 1970). Several risings were used to remove the acid from the spores before using or storing.

Pine Inoculation

Discs of basidiospores may be stored for several weeks; however, in our case spores less than a week old were used in all of the pine inoculations. A water suspension of basidiospores is prepared by washing the spores from the discs into a beaker of water. The inoculum density of the spore solution was determined using modified methods of those developed by Dwinell (Matthews et al, 1971) employing a Coulter Electronic Particle Counter.

All the seedlings were inoculated at 6 weeks of age with a precisely controlled mechanical spray inoculation system. The system basically consists of two fine mist atomizer sprayers and a conveyor (Fig. 1). The conveyor speed is controlled at 6 ft./min. and volume passing through both spray nozzles is standardized at 5 mls. of spore suspension per 10-second exposure time period. It takes exactly 10 seconds for the seedling tray to pass completely past the spray nozzles. A regulated

air pressure-flow meter system is used to control the volume of air passing through the nozzles for atomization.

The volume of spray passing through the sprayers must be verified at the 5 mls./10 second rate at the onset of the day of the inoculations.

As the seedling trays come off the conveyor belt, they are placed immediately in an incubation chamber for 24 hours where relative humidity is maintained at above 96 percent and temperature at 69° to 71°F.

A schematic diagram of the chamber is presented in Figure 2. The box itself is merely a grocery store type walk-in refrigerated cooler minus the refrigeration system. Inside the box is a sheet metal cone roof with a perforated pipe (D) around its exterior perimeter. This roof is held up by redwood posts. Shelving is attached to the posts. Burlap curtain (C) hangs down from the sheet metal roof's perimeter and is affixed so that water dripping from the perforated pipe keeps the curtain constantly saturated. Water dripping from the curtain falls to chamber floor and collects to form a 2- to 4-inch deep pool over the entire floor surface. A pallet floor extends over the pool. The floor not only offers a relatively firm surface for footing while placing seedling trays on the shelves but also space to place further trays once the shelves are filled. Constant evaporation from the burlap and water pool on the floor is the main means of keeping the chamber near saturated humidity. A humidifier, controlled by an electric timer set at 3 minutes on 3 minutes off cycle, further supplements the amount of moist air. The humidifier is attached to the same water system (D) as is the water dripping down the curtain to the chamber floor. The temperature of the water flowing through the whole system is maintained by a Blue M Magni Whirl water bath (Model #MR-3240C-1, Blue M Electric Co., Blue Island, Ill.), which maintains the water at 69°F. plus or minus one degree (A). Two pumps are used to circulate the water, one from a sump (B) to the water bath and the other from water bath into the pipe circulation system (D). The efficiency of this box is checked and monitored repeatedly using a Psychon psychrometer and several types of thermometers and weather recorders.

Pine Culture

One of the most important facets of the standardization of this evaluation is that of the pine culture.

The pines were grown throughout the evaluation in a glass-enclosed greenhouse, Temperatures rarely exceeded 90°F. and then only for a brief period of 1 to 2 hours. Night temperatures rarely dropped below 40°F. and were normally above 50°F. A standardized day (16 hours) was accomplished by supplementing natural light with wide spectrum very high output Sylvania Gro-Lux fluorescent lamps.

The soil was analyzed by North Carolina State University soil scientist Dr. Charles Davey. Based on his findings, the seedling trays were fertilized to bring the soil up to standards for good growth. The soil was sterilized using a Famco Electric Sterilizer.

Each seedling was insured an approximately equal growing space with a planting dowel containing 20 evenly spaced pegs to establish seedling location in the tray's soil before insertion of the seedlings. The pine seed was germinated in vermiculite beds under a mist-a-matic or timed spray system. The seedlings were transplanted to the planting trays as soon as they germinated and before they shed their seed coats.

Evaluation

Evaluation of the seedlings consisted of checking the seedlings for galls at 6 months. Only trees exhibiting definite active stem swellings were counted as successfully infected. Healed over and non-active galls are counted as resistant.

Results

The results for the 1971 loblolly are expressed in Table 1. The results for the 1972 loblolly and slash are expressed in Tables 2 and 3, respectively.

The last column is the mean infection obtained for that particular seed lot in our previous tests at the testing center using the higher densities.

For the 1971 loblolly seed lots, 70,000 spores/ml seems ideal. The intermediate resistance masked at 150,000 spores/ml becomes readily apparent. In the 1972 test, 70,000 or 85,000 gave results superior to the previously used 150,000. Of note was reduction in infection of some of the 1972 seed lots from the 1971 seed lots. A possible explanation offered is that the rouging of the seed orchards of many susceptible families after the 1971 seed collection resulted in a reduction in the susceptibility of the male parents.

The slash seed lot test, unfortunately, contained only one seed lot previously tested. However, at 25,000 and 10,000 spores/ml, the intermediate family masked at higher densities becomes apparent.

Conclusion

Based on these results, we are presently using 70,000 spores/ml for loblolly and 25,000 spores/ml for slash. Forest Service research has initiated projects on inoculum density and based on their results further adjustments may be made in the future.

Table 1. Loblolly Inoculum Density Test I (1971 Seed).

Family	Percent Infection by Inoculum Density Level (Spores per Milliliter)						
	25,000	55,000	70,000	85,000	100,000	150,000	Previous 150,000*
Livingston Parish	20.6	50.7	65.7	58.5	69.4	73.2	--
Resistant (1)*	45.8	51.5	57.8	63.8	72.3	71.5	76.0
Intermediate (1)*	39.8	58.0	72.4	79.8	74.3	91.0	86.0
Intermediate (2)*	41.9	79.0	74.4	85.4	84.2	94.3	95.0
Commercial	49.0	85.4	90.3	94.5	95.1	94.1	--

*Previous test ratings: Percent infected at 6 months: 150,000 spores/ml; mean of 25 replicate trays of 20 seedlings each (Laird, Knighten, and Wolfe, 1973a).

Table 2. Loblolly Inoculum Density Test II (1972 Seed)

Family	Percent Infection by Inoculum Density Level (Spores per Milliliter)						
	25,000	55,000	70,000	85,000	100,000	150,000	Previous 150,000*
Livingston Parish	15.1	34.4	51.7	59.4	60.7	64.4	--
Resistant (1)*	30.0	47.6	48.8	53.8	58.2	57.0	76.0
Resistant (2)*	25.0	43.7	53.5	60.0	54.8	54.3	65.0
Intermediate (1)*	30.6	47.7	65.0	64.4	75.6	76.2	86.0
Susceptible (1)*	33.8	50.7	72.5	84.4	80.1	80.5	96.0
Susceptible (2)*	40.1	68.2	81.3	89.7	85.7	93.2	91.0

*Previous test ratings: Percent infected at 6 months; 150,000 spores/ml.; mean of 25 replicate trays of 20 seedlings each (Laird, Knighten, and Wolfe, 1973a).

Table 3. Slash Pine - Inoculum Density Test.

Family	Percent Infection by Inoculum Density Level (Spores per Milliliter)						
	10,000	25,000	40,000	55,000	70,000	75,000	Previous 150,000*
Resistant	4.9	19.2	24.4	34.3	49.8	50.1	--
Intermediate	43.8	79.4	87.9	87.9	89.8	90.0	--
Susceptible*	63.5	92.5	94.3	95.7	96.3	98.2	97.0
Commercial lot	59.3	81.0	81.8	93.4	94.3	92.3	--

Data consists of eight replicate trays of 20 seedlings each divided into 2 runs a month or more apart (total 160 seedlings).

*Previous test ratings: Percent infected at 6 months; 75,000 spores/ml; mean of 25 replicate trays of 20 seedlings each (Laird, Knighten, and Wolfe, 1973).

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